

FORM PTO-1390 (REV. 9-2001)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER BSWV-P01-002	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	
				10/018127	
INTERNATIONAL APPLICATION NO. PCT/AU00/00643		INTERNATIONAL FILING DATE 07 June 2000		PRIORITY DATE CLAIMED 07 June 1999	
TITLE OF INVENTION A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS					
APPLICANT(S) FOR DO/EO/US CLANCY, Robert Llewellyn; GLEESON, Maree					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</p> <p>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p>a. <input type="checkbox"/> is attached hereto.</p> <p>b. <input checked="" type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</p> <p>b. <input type="checkbox"/> have been communicated by the International Bureau.</p> <p>c. <input checked="" type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (1 p., unsigned)</p> <p>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11 to 20 below concern document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p>14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>15. <input type="checkbox"/> A substitute specification.</p> <p>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</p> <p>18. <input checked="" type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</p> <p>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</p> <p>20. <input checked="" type="checkbox"/> Other items or information:</p>					

U.S. APPLICATION NO. 10/018127		INTERNATIONAL APPLICATION NO. PCT/AU00/00643		ATTORNEY'S DOCKET NUMBER BSWV-P01-002	
---------------------------------------	--	--	--	---	--

21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY <div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 0 auto;">\$ 740</div>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	36 - 20 =	16	x \$18.00	\$	288
Independent claims	3 - 3 =	0	x \$84.00	\$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$280.00	\$	280
TOTAL OF ABOVE CALCULATIONS =				\$	1,308
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	
SUBTOTAL =				\$	654
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	654
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$	
				Amount to be refunded:	\$
				charged:	\$ 654

a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **18-1945** in the amount of \$ **654** to cover the above fees. A duplicate copy of this sheet is enclosed.

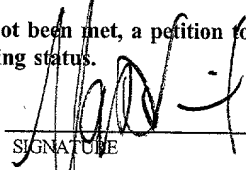
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **18-1945**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

PATENT GROUP
Ropes & Gray
One International Place
Boston, MA 02110
U.S.A.


 SIGNATURE
Matthew P. Vincent
 NAME
36,709
 REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Robert Llewellyn Clancy, :
Maree Gleeson. :
:Docket No.: BSWV-P01-002
Appln. No. 35 USC 371 of :
PCT/AU00/00643 :
International :
Filing Date: 07 June 2000 :
For: A METHOD OF DETERMINING POTENTIAL
SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

BOX PCT (DO/EO/US)
Assistant Commissioner for Patents
Washington, D.C. 20231

Certificate of Express Mailing (37 CFR 1.10)Label No. EL920 874 515US

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and is addressed to the Assistant Commissioner of Patents; BOX PCT (DO/EO/US), Washington, D.C. 20231, on the date set forth below.

7 December 2001

Date of Signature and of Mail Deposit

Printed name: Michael Crowley

PRELIMINARY AMENDMENT

Dear Sir:

Preliminary to examination on its merits, kindly amend the above-referenced patent application as follows:

In the Specification:

Page 1, line 1, after the title and before "Technical Field" please insert:

--This application is the U.S. national phase application of, and claims priority from, PCT/AU00/00643, international filing date 07 June 2000, and from Australia PQ 0810, filed 07 June 1999, the specifications of which are incorporated herein by reference.--

In the claims:

Please cancel existing claims 1-19 and add the following new claims:

- 20. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:
- (a) determination of the immunoglobulin A (IgA) level in a sample from the subject; and
 - (b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.
21. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:
- (a) determination of immunoglobulin A1 (IgA1) level in a sample from the subject; and
 - (b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.
22. A method according to claim 20 or claim 21 wherein the subject is a human infant.
23. A method according to claim 20 or claim 21 wherein the sample is a sample from a subject at the time of, or any time up to approximately 2 weeks after, an upper respiratory tract infection (URTI) and/or symptoms.
24. A method according to claim 20 or claim 21 wherein the immunoglobulin is secretory immunoglobulin.
25. A method according to claim 20 or claim 21 wherein the immunoglobulin is salivary immunoglobulin.
26. A method according to claim 20 or claim 21 wherein the sample is whole unstimulated saliva.

27. A method according to claim 20 or claim 21 wherein the subject is not fasting when the sample is collected.
28. A method according to claim 20 or claim 21 wherein the immunoglobulin level is determined by ELISA.
29. A method according to claim 20 or claim 21 wherein the immunoglobulin level is determined by radial immunodiffusion.
30. A method according to claim 20 or claim 21 wherein the immunoglobulin level is analysed by a rapid near-subject assay.
31. A method according to claim 20 or claim 21 wherein the immunoglobulin level is determined by contacting a body secretion with an assay device or system on a support.
32. A method according to claim 20 or claim 21 wherein the immunoglobulin level is analysed by contacting an assay device or system with the saliva of the subject *in situ*.
33. A method according to claim 20 or claim 21 wherein the standard is a normal population standard.
34. A method according to claim 20 or claim 21 wherein the standard is an internal personal standard.
35. A method according to claim 20 or claim 21 further including comparison of the ratio of immunoglobulin level to other indices.
36. A method according to claim 20 or claim 21 further including comparison of the ratio of immunoglobulin level to other indices selected from the group consisting of IgM, IgG, acute phase reactants and other cellular components.

37. A method for assessing potential susceptibility to development of ALTE and/or SIDS in an infant including:

- (a) determination of the immunoglobulin A (IgA) and/or immunoglobulin A1 (IgA1) level in a sample of the infant's whole, unstimulated saliva; and
- (b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA and/or said IgA1 level with a predetermined standard.

38. A kit when used in a method according to any one of claims 20, 21, or 37. --

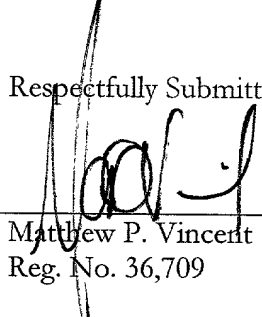
REMARKS

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Applicants hereby request that any fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945**.

Date: 07 December 2001

Customer No: 28120
Ropes & Gray
One International Place
Boston, MA 02110

Respectfully Submitted,


Matthew P. Vincent
Reg. No. 36,709

**A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO
DEVELOPMENT OF ALTE AND/OR SIDS**

TECHNICAL FIELD

5 The present invention relates to methods for determining predisposition to acute life threatening episodes (ALTE) and/or sudden infant death syndrome (SIDS) and in particular to methods of assessing potential susceptibility to development of ALTE and/or SIDS by determining a subject's total IgA and/or IgA1.

BACKGROUND

10 A great deal has been done to minimize the risk of SIDS by non-specific methods related to infant care. However, prevention using specific assays related to causal mechanisms has not been explored. Identifying a causal mechanism may be expected to make a major impact on SIDS outcome through general awareness, and if used in conjunction with non-specific nursing care. The development of new techniques for
15 identifying infants at risk of SIDS could be a significant outcome.

Interest in this approach to the prevention of SIDS arose as a result of an unusual opportunity of observing a 'prospective' case of SIDS during a study of 250 normal infants [1]. The infants were followed from birth, measuring parameters of immune status in saliva. The key observation in the one child who died from SIDS was an
20 extraordinarily high IgM level appearing after a mild respiratory tract infection, several weeks before the child suddenly died. While all parameters tested in the SIDS victim (i.e. albumin, IgG and IgA) were in excess of the 90th percentile level, relative levels of IgM were the highest, being more than three times the level of the 90th percentile figure (compared to approximately one and a half times the 90th percentile level for albumin,
25 IgG and IgA). This observation in a single case was consistent with post mortem studies

showing large numbers of IgM containing plasma cells in the trachea and gut of subjects dying from SIDS [2-5]. The level of IgM was much in excess of any small increases seen in matched infection control studies [6]. These observations raised the possibility that assay of IgM in saliva of infants with an upper respiratory tract infection may be a very useful marker of risk of SIDS, reflecting the disturbed mucosal immunoregulation that underpins the risk.

The numerous epidemiological studies of SIDS have identified many of the risk factors of SIDS but have failed to find a cause [7]. The role of infection and disturbed immunity has been proposed as one of the potential mechanisms for SIDS [8]. The common findings at autopsy of SIDS infants are consistent with infection or inflammation as a contributing cause of death [9]. SIDS has been reported to occur after a mild upper respiratory tract infection (URTI) [9-12], however there is no evidence that favours infections by any virulent pathogen. A low grade pathogen, that results in overstimulation of the immune system may be one important link in the chain of events that culminates in respiratory arrest.

There is evidence from post mortem studies [2-5, 13-14] and a prospective case study [1] of a gross disturbance of mucosal immunity in SIDS associated with prior respiratory illness or inflammation. These studies suggest an infective agent is responsible for the disturbance observed in the immune parameters which thus provides a clinical "trigger" for testing the infant for risk..

Infants presenting with episodes of apnoea from which the infant recovers are termed acute life threatening episodes (ALTEs) and are classified as "near-miss" SIDS when no underlying medical condition is identified. ALTE children could, therefore, be

expected to have a similar pattern of dysregulation of mucosal immunity to SIDS children.

To date, however, no method exists by which a prediction of the potential susceptibility to development of ALTE and/or SIDS can be carried out on the basis of a
5 specific immunological assay.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

As indicated above, the prior art [1] pointed towards IgM levels after URTI
10 infection as being a potentially useful parameter for study in ALTE/SIDS research. However, it has been unexpectedly found that IgA levels were significantly and consistently higher in ALTE or "near miss" SIDS cases. IgA can therefore be used as a predictor of susceptibility to development of ALTE and/or SIDS.

According to a first aspect, the present invention provides a method of assessing
15 potential susceptibility to development of ALTE and/or SIDS in a subject including:

- a) determination of the immunoglobulin A (IgA) level in a sample from the subject;
and
- b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.

20 According to a second aspect, the present invention provides a method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

- a) determination of immunoglobulin A1 (IgA1) level in a sample from the subject;
and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.

Preferably, the subject is a human infant.

Preferably, the sample is a sample from a subject at the time of, or any time up to
5 approximately 2 weeks after, an upper respiratory tract infection (URTI) or symptoms.

Preferably, the immunoglobulin is secretory immunoglobulin. Preferably,
the secretory immunoglobulin is salivary immunoglobulin. Preferably, the sample is
whole unstimulated saliva. However, it will be clear to the skilled addressee that other
body secretions known to contain IgA and/or IgA1 would also be useful as samples for
10 the present method.

Preferably, the subject is not fasting when the sample is collected.

Preferably, the immunoglobulin level is determined by ELISA. However, it will
be understood that the immunoglobulin level may be determined by radial
immunodiffusion and/or similar methods, all of which would be known to a skilled
15 addressee. The method is particularly suitable for an assay in which the immunoglobulin
level is analysed by a rapid, near-subject assay. It can thus provide a yes/no test for
immediate action.

In one embodiment, the immunoglobulin level is determined by contacting a body
secretion with an assay device or system on a support. The sample need not necessarily
20 be removed from the subject but the method may be applied *in situ*. For example, the
immunoglobulin level may be analysed by contacting an assay device or system with the
saliva of the subject *in situ*.

The predetermined standard may be any appropriate standard, for example, a normal population standard or an internal personal standard. The skilled addressee will recognize the types of standards which will be useful in the present invention.

In a third aspect, the present invention provides a method for assessing potential
5 susceptibility to development of ALTE and/or SIDS in an infant including:
(a) determination of the immunoglobulin A (IgA) and/or immunoglobulin A1 (IgA1) level in a sample of the infant's whole, unstimulated saliva; and
(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA and/or said IgA1 level with a predetermined standard.

10 It will be clear to the skilled addressee that the determination of the level of IgA or IgA1 could also be used as a predictor of susceptibility to development of ALTE and/or SIDS in conjunction with other indices such as other immunoglobulins, for example IgM or IgG, acute phase reactants or cellular components.

In a fourth aspect, the present invention provides a kit when used in a method
15 according to any one of the first to third aspects.

In the context of the present invention, the word "standard" includes within its meaning, but is not limited to, the normal population level of immunoglobulin ie. the average IgA or IgA1 value for age-matched normal subjects. It may also be an internal personal standard i.e. the level of IgA or IgA1 in a sample taken previously from the
20 same individual.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 IgA concentration levels (mg/L) for ALTE (■), "mild" (○) and well (▲) infants - initial sample.

- Figure 2 IgM concentration levels (mg/L) for ALTE (■), "mild" (○) and well (▲) infants - initial sample.
- Figure 3 IgG concentration levels (mg/L) for ALTE (■), "mild" (○) and well (▲) infants - initial sample.
- 5 Figure 4 IgA concentration levels (mg/L) for ALTE (■), "mild" (○) and well (▲) infants - 14 day sample. Subject RO3 was assessed 12 days post infection.
- Figure 5 IgM concentration levels (mg/L) for ALTE (■), "mild" (○) and well (▲) infants - 14 day sample. Subject RO9 was assessed 14 days post immunisation with triple antigen and *Haemophilus influenzae* B.
- 10 Figure 6 IgG concentration levels (mg/L) for ALTE (■), "mild" (○) and well (▲) infants - 14 day sample.
- Figure 7A IgA1 concentration levels (mg/L) for ALTE, "mild" and well infants.
- Figure 7B Data of Figure 7A shown with 95% confidence intervals.
- Figure 8 Total IgA concentration levels (mg/L) for ALTE, "mild" and well infants in which IgA1 concentration levels (mg/L) is shown in Figures 7A and 7B.
- 15

DESCRIPTION OF THE INVENTION

A preferred embodiment of the invention will now be described, by way of example only.

EXAMPLE 1

20 Total IgA, IgG and IgM levels in Saliva of Infants with ALTE

Saliva Collection

Whole mixed saliva was collected by gentle suction from the buccal cavity of the mouth [15]. This technique is successful in children (aged from 1 day) and adults [1,16].

Questionnaire

A standardised questionnaire was used to collect the relevant SIDS related demographics. The classification into the “near-miss” SIDS group (ALTE) was made by the attending paediatrician on the basis of clinical investigations.

5 Saliva Tests

Salivary immunoglobulins were measured by ELISA and albumin by rate nephelometry (Beckman, ARRAY) [16].

Statistical Analysis

The differences in mucosal immune parameters was determined between the
10 ALTE infants and two control groups of subjects (mild URTI and well infants) using analysis of variance (ANOVA) or the appropriate non-parametric statistics.

Subjects

37 subjects aged 1-10 months were recruited (20 males, 17 female) in 3 categories:

- Acute life Threatening episodes (ALTE) at John Hunter Hospital (n=5)
- 15 • Mild respiratory tract illness (MILD) from General Practitioners (n=11)
- A well control group (WELL) from immunisation clinics (n=21).

Questionnaire Data

- There were more males (n=4) than females (n=1) in the ALTE group.
- There were no significant differences between the groups for age, birth history, family
20 demographics, ethnic background or family history of SIDS.
- There were a higher percentage of children exposed to passive tobacco smoke (60%, n=3) in the ALTE group compared to the MILD (36%, n=4) and WELL (10%, n=2) control groups (p=0.03).

- The ALTE group had a higher percentage of families in the average-below average socio-economic category (100%) compared to the other control groups ($p < 0.01$).
 - There were no differences between the groups for feeding history, immunisation status, sleeping position.
- 5 • In 4 of the 5 ALTE subjects an Upper Respiratory Tract Illness (URTI) was suspected as the cause of the ALTE (Table 1).

TABLE 1							
Doctor Questionnaire - ALTE							
	IgA (mg/L)		Q12	Q32	Q34	Q40	Follow Up
Study Number	Initial	14 Day	Face Covered	Prior URTI	Passive Smoke	URTI Suspected	Suspected Clinical Cause
A01	115.5	56.1	N	N	Y	N	Gastro-esophageal reflux
A02	228.9	22.8	N	Y	Y	Y	RSV+ve Bronchiolitis
A03	410.6	230.9	N	Y	Y	Y	RSV+ve Bronchiolitis
A04	91.0	28.9	Y	Y	N	Y	RSV+ve Bronchiolitis
A05	26.6	79.2	N	Y	N	Y	Reflux with aspiration

Salivary Immunoglobulins

Two samples of saliva were collected from each subject. The first sample was collected from the ALTE group within 24 hours of admission to hospital and from the MILD respiratory illness group within 48 hours of presentation of their General Practitioners. The WELL group were recruited from immunisation clinics and saliva

collected at ages to approximate the ages of presentation of the ALTE and MILD groups.

The second sample was collected 14 days later from each subject.

The figures in Appendix C have the age related 5th-95th percentile reference ranges indicated for each salivary immunoglobulin over the first year of life.

- 5 • The salivary IgA, IgG and IgM concentration in the ALTE group were all significantly higher than the MILD (Tables 2A and 2B) and WELL (Tables 3A and 3B) groups for both sample 1 and 2 (Figures 1 and 2).
- There were no significant differences between the MILD and WELL groups for either sample 1 or sample 2 (Tables 2C and 3C).
- 10 • There were two subjects in the MILD group who had grossly elevated salivary immunoglobulin concentrations in the 14 day collections. (See Appendix C).
- RO3 had an elevated IgA 12 days post infection.
- RO9 had an elevated IgM that is most likely accounted for by immunisation with Triple antigen and *Haemophilus influenzae* B 14 days prior to the saliva collection.
- 15

TABLE 2A First Sample Analysis of Immunoglobulins - ALTE vs MILD							
	ALTE			MILD			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	115.55	(27-411)	11	9.93	(0-37)	<0.01
IgG	5	9.21	(0-16)	11	0.00	(0-3)	0.02
IgM	5	4.61	(3-24)	11	2.18	(0-16)	0.04

TABLE 2B First Sample Analysis of Immunoglobulins - ALTE vs WELL							
	ALTE			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	115.55	(27-411)	21	11.37	(0-67)	<0.01
IgG	5	9.21	(0-16)	21	0.00	(0-8)	0.01
IgM	5	4.61	(3-24)	21	1.00	(0-33)	0.01

TABLE 2C First Sample Analysis of Immunoglobulins - MILD vs WELL							
	MILD			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	11	9.93	(0-37)	<0.01	11.37	(0-67)	0.68
IgG	11	0.00	(0-3)	0.02	0.00	(0-8)	0.66
IgM	11	2.18	(0-16)	0.04	1.00	(0-33)	0.36

TABLE 3A Second Sample Analysis of Immunoglobulins - ALTE vs MILD							
	ALTE			MILD			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	56.06	(23-231)	11	8.88	(1-255)	0.04
IgG	5	2.99	(2-7)	11	0.00	(0-4)	0.03
IgM	5	9.39	(2-16)	11	2.31	(0-27)	0.07

TABLE 3B Second Sample Analysis of Immunoglobulins - ALTE vs WELL							
	ALTE			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	56.06	(23-231)	20	10.53	(0-58)	<0.01
IgG	5	2.99	(2-7)	20	0.00	(0-6)	<0.01
IgM	5	9.39	(2-16)	20	1.66	(0-14)	<0.01

TABLE 3C Second Sample Analysis of Immunoglobulins - ALTE vs WELL							
	MILD			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	11	8.88	(1-255)	20	10.53	(0-58)	0.71
IgG	11	0.00	(0-4)	20	0.00	(0-6)	0.75
IgM	11	2.31	(0-27)	20	1.66	(0-14)	0.56

Conclusions

- 5 • The grossly elevated salivary IgA concentration in 4 of 5 ALTE subjects at presentation was not observed in the MILD or WELL control groups. Salivary IgA can therefore act as a marker for ALTE (and SIDS) in subjects presenting with an otherwise mild respiratory illness. This was an unexpected result since the prior art [1] suggested IgM would be the most useful parameter in prediction of ALTE/SIDS susceptibility.

- The elevated salivary IgA and IgM concentrations in 4 of 5 ALTE support the concept of an infection or inflammatory cause in ALTE (and SIDS).
- RSV positive Bronchiolitis was evident in 3 of 5 ALTE subjects.

EXAMPLE 2

5 Total IgA and IgA1 subclass in Saliva of Infants with ALTE

Study Groups

Saliva samples were collected from infants on the day of admission to hospital for an unexplained acute life-threatening episode (ALTE). The infants were included in this study if all congenital or obstructive causes of apnoea had been excluded. This
10 group of subjects have been classified as the "near-miss SIDS" infants.

Saliva was collected from age matched control subjects in two categories. Normal healthy infants were recruited from the Child Immunisation Clinics and classified as WELL infants. The second group was recruited from general practitioners, who referred infants with a mild upper respiratory infection and these infants were
15 classified as the MILD infection control group. Saliva was collected on the day of referral with the mild infection.

Laboratory Analysis

Saliva samples were assayed by an Enzyme Linked Immunosorbant Assay (ELISA) to detect total IgA and IgA1 subclass antibodies. The assay uses a WHO/IUIS
20 approved monoclonal antibody for IgA1 subclass as the capture antibody in conjunction with a polyclonal antibody-enzyme labelled detection system.

Results

The results indicate that the concentrations of IgA1 subclass in the saliva from infants suffering an ALTE were significantly higher than the concentrations for the infants in the control groups of normal healthy infants and those suffering a mild upper respiratory infection ($p=0.009$) (Table 4 and Figures 7A and 7B).

The concentrations of IgA1 in saliva from the normal healthy infants were not significantly different from those with mild respiratory illnesses.

Five samples were assayed from each of the three study groups: ALTE babies, babies with mild infection, and well babies. The level of IgA1 was generally much higher in the samples from the ALTE babies compared to the levels in the other two groups ($p=0.009$). Levels in the mild infection and well baby groups were similar.

Statistical Analysis

The non-parametric Kruskal-Wallis test was used to compare the distributions of IgA1 values for the three groups. The probability of the three sample groups having equal IgA1 distributions is $p=0.009$. Due to small sample sizes, the estimated 95% confidence intervals about the group medians are equivalent to the range (ie. min, max) of the data.

Table 1

group	IgA1 (mg/L)		
	min	max	median
ALTE	12.27	96.35	68.35
Mild infection	2.79	9.78	9.92
Well	3.02	14.85	7.29

Total IgA levels were also elevated in the same infants who took part in this study (Figure 8).

Conclusion

IgA1 concentrations are significantly elevated in infants suffering an unexplained
5 ALTE (4 out of 5 children). Three out of the same 5 children with ALTE were found to
have elevated total IgA levels. Therefore, although it is clear that both IgA and IgA1 are
useful parameters in the prediction of ALTE, IgA1 levels may be the more useful
parameter. Since ALTE are classified as "near-miss" SIDS (when no other medical
condition is identified), it follows that both IgA and IgA1 are also useful parameters in
10 the prediction of SIDS.

Although the invention has been described with reference to specific examples, it
will be appreciated by those skilled in the art that the invention may be embodied in
many other forms.

REFERENCES

1. Gleeson M, Clancy RL, Cripps AW (1993) Mucosal immune response in a case of sudden infant death syndrome. Paed Res Vol 33:No 6 554-556.
2. Thrane PS, Rognum TO, Brandtzaeg P (1990) Increased immune response in upper respiratory and digestive tracts in SIDS. Lancet 1:229-230.
3. Forsyth KD, Weeks SC, Koh L, Skinner J, Bradley J (1989) Lung immunoglobulins in the sudden infant death syndrome. British Medical Journal 298:23-26.
4. Stolenberg L, Saugstad OD, Rognum TO (1992) Sudden infant death syndrome victims show local immunoglobulin M response in tracheal wall and immunoglobulin. A response in duodenal mucosa. Paed Res 31:372-375.
5. Guntheroth WG (1989) Interleukin-1 as intermediary causing prolonged sleep apnea and SIDS during respiratory infections. Med Hypotheses 28:121-123.
6. Gleeson M, Dobson AJ, Firman DW, Cripps AW, Clancy RL, Wlodarczyk JH, Hensley MJ (1991) The variability of immunoglobulins and albumin in salivary secretions of children. Scand J Immunol. 33: 533-541.
7. Wilkinson M, Jones C (1997) Australian SIDS statistics. Australia SIDS Conference. Abstract 301.
8. Blackwell CC, Weir DM, Busuttill A (1997) Infectious Agents and SIDS: Analysis of risk factors and preventive measures. SIDS and Infant Mortality. (In press).
9. Byart R (1997) Does SIDS exist anymore? Australian SIDS Conference. Directions of SIDS Research, Presentation.
10. Shannon DC, Kelly DH (1982) SIDS and near-SIDS. N. Engl J Med 306:959-965.

11. Hoffman JH, Damus K, Hillman L, Krongrad E (1988) Risk factors for SIDS. Ann NY Acad Sci 533:13-20.
12. Nelson EA, Taylor BJ, Mackay SC (1989) Childcare practices and the sudden infant death syndrome. Aust Paed J 25:202-204.
- 5 13. Naeye RL (1990) Preventing the sudden infant death syndrome. Paediatr Perinat Epidemiol 4:12:21.
14. Ogra PL, Ogra SS, Coppola PR (1975) Secretory component and sudden infant death syndrome. Lancet 2:387-390.
15. Ostergaard PA, Blom M (1977) Whole saliva immunoglobulin levels in 60 healthy
10 children determined by a sensitive electroimmuno technique after prior carbamylation.
Jnr Clin Chem Clin Biochem 15:393-6.
16. Gleeson M, McDonald WA, Cripps AW, Pyne DB, Clancy RL, Fricker PA, Wlodarczyk JH (1995) Exercise, stress, and mucosal immunity in elite swimmers. In Advances in Mucosal Immunology. Ed J. Mestecky et al. Plenum Press New York 571-
15 574.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:
 - a) determination of the immunoglobulin A (IgA) level in a sample from the subject;
 - 5 and
 - b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.
2. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:
 - 10 a) determination of immunoglobulin A1 (IgA1) level in a sample from the subject; and
 - b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.
3. A method according to claim 1 or claim 2 wherein the subject is a human infant.
- 15 4. A method according to any one of claims 1 to 3 wherein the sample is a sample from a subject at the time of, or any time up to approximately 2 weeks after, an upper respiratory tract infection (URTI) and/or symptoms.
5. A method according to any one of claims 1 to 4 wherein the immunoglobulin is secretory immunoglobulin.
- 20 6. A method according to claim 5 wherein the secretory immunoglobulin is salivary immunoglobulin.
7. A method according to claim 6 wherein the sample is whole unstimulated saliva.

8. A method according to any one of claims 1 to 7 wherein the subject is not fasting when the sample is collected.
9. A method according to any one of claims 1 to 8 wherein the immunoglobulin level is determined by ELISA.
- 5 10. A method according to any one of claims 1 to 8 wherein the immunoglobulin level is determined by radial immunodiffusion.
11. A method according to any one of claims 1 to 9 wherein the immunoglobulin level is analysed by a rapid near-subject assay.
12. A method according to any one of claims 1 to 11 wherein the immunoglobulin
10 level is determined by contacting a body secretion with an assay device or system on a support.
13. A method according to any one of claims 1 to 12 wherein the immunoglobulin level is analysed by contacting an assay device or system with the saliva of the subject *in situ*.
- 15 14. A method according to any one of claims 1 to 13 wherein the standard is a normal population standard.
15. A method according to any one of claims 1 to 13 wherein the standard is an internal personal standard.
16. A method according to any one of claims 1 to 15 further including comparison of
20 the ratio of immunoglobulin level to other indices.
17. A method according to claim 16 wherein the other indices are selected from the group consisting of IgM, IgG, acute phase reactants or cellular components.

18. A method for assessing potential susceptibility to development of ALTE and/or SIDS in an infant including:
- (a) determination of the immunoglobulin A (IgA) and/or immunoglobulin A1 (IgA1) level in a sample of the infant's whole, unstimulated saliva; and
 - 5 (b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA and/or said IgA1 level with a predetermined standard.
19. A kit when used in a method according to any one of claims 1 to 18.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/75656 A1

(51) International Patent Classification⁷: G01N 33/53, 33/68

(74) Agent: BALDWIN SHELSTON WATERS; 60 Margaret Street, Sydney, NSW 2000 (AU).

(21) International Application Number: PCT/AU00/00643

(22) International Filing Date: 7 June 2000 (07.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 0810 7 June 1999 (07.06.1999) AU

(71) Applicant (for all designated States except US): THE UNIVERSITY OF NEWCASTLE RESEARCH ASSOCIATES LIMITED [AU/AU]; Industry Development Centre, University Drive, Callaghan, NSW 2308 (AU).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventors; and

(75) Inventors/Applicants (for US only): CLANCY, Robert [AU/AU]; 11 High Street, Newcastle, NSW 2300 (AU). GLEESON, Maree [AU/AU]; 202 Merewether Street, Merewether, NSW 2291 (AU).

WO 00/75656 A1

(54) Title: A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

(57) Abstract: The present invention relates to methods for determining predisposition to apparent life-threatening events (ALTE) (also referred to as "acute life threatening episodes") and/or sudden infant death syndrome (SIDS) and in particular to methods of assessing potential susceptibility to development of ALTE and/or SIDS by determining a subject's total IgA and/or IgA1 level.

1/9

Figure 1

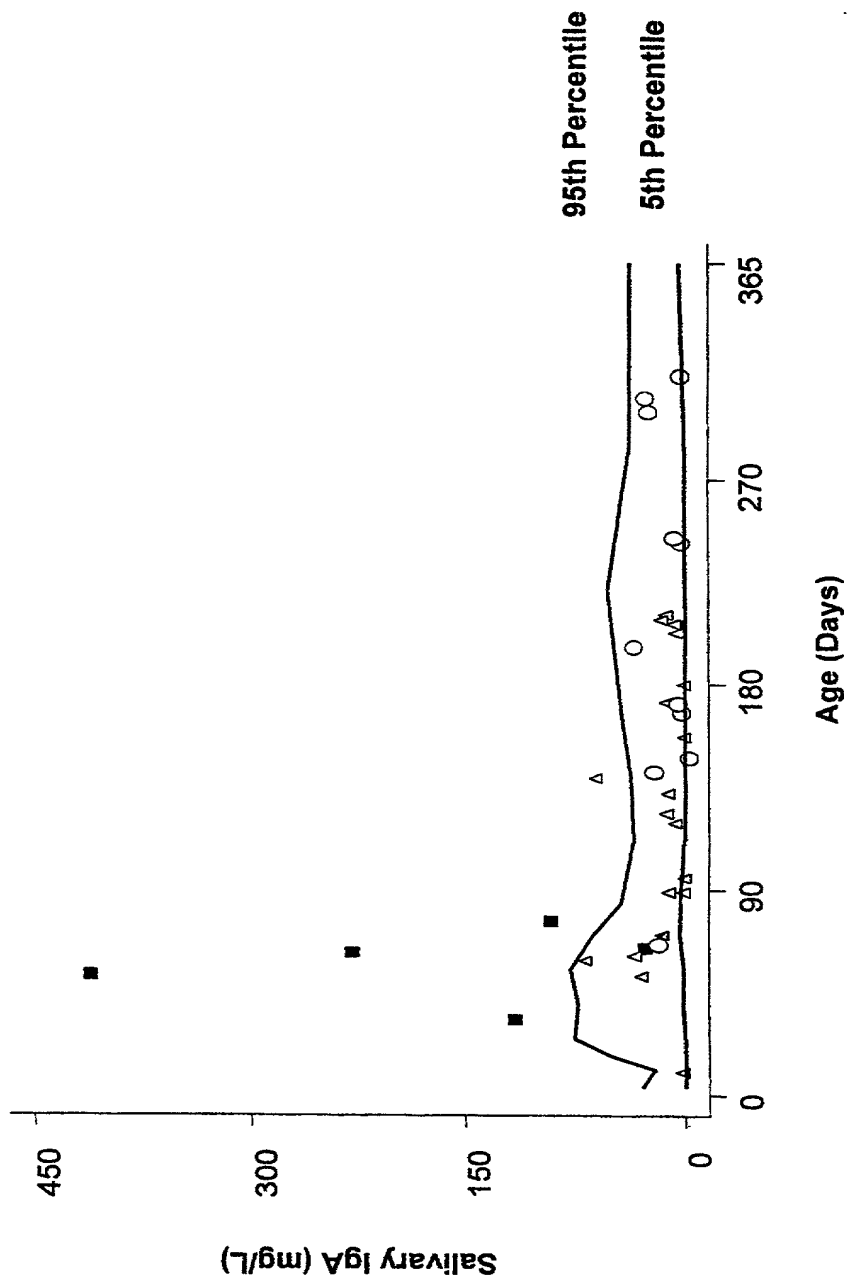


Figure 2

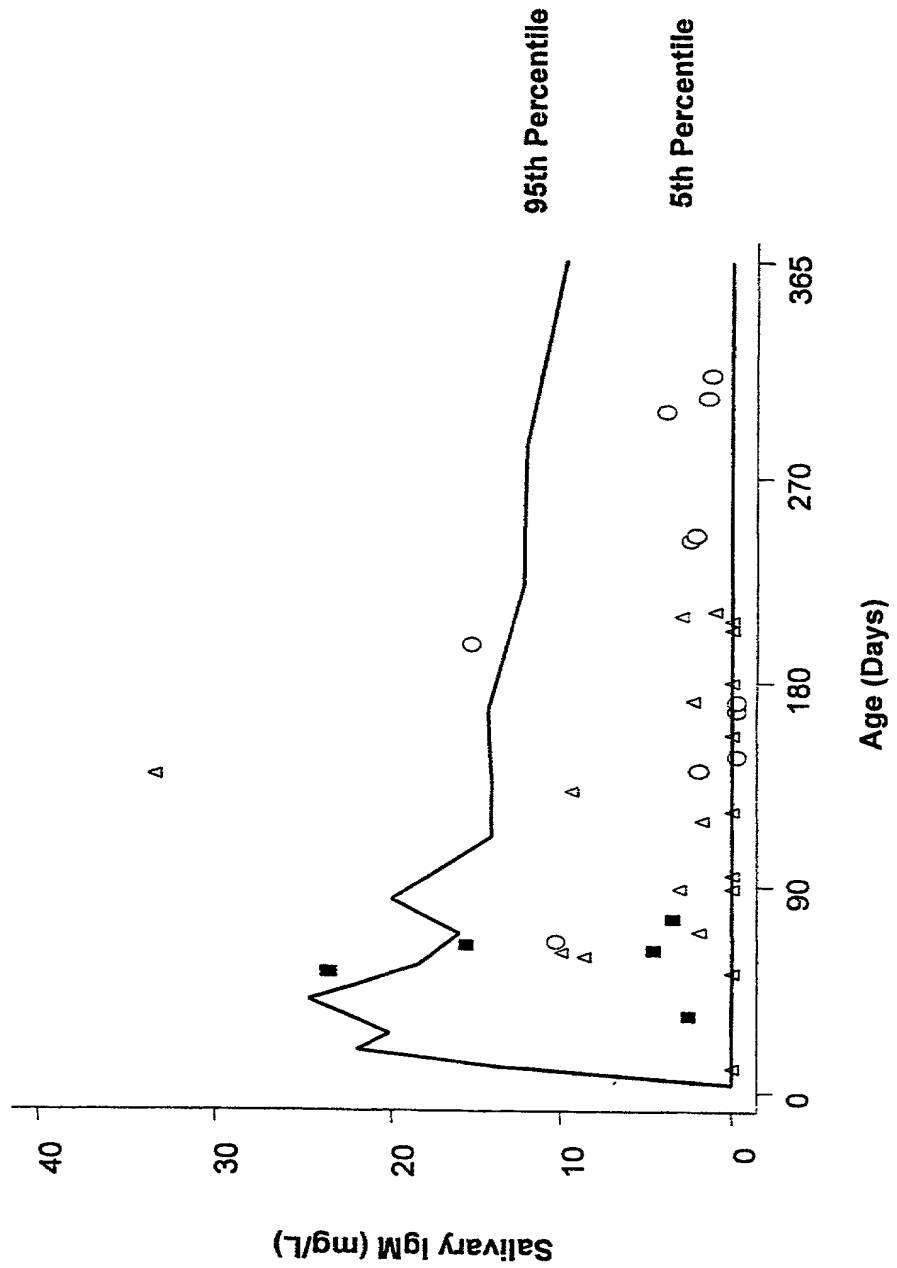


Figure 3

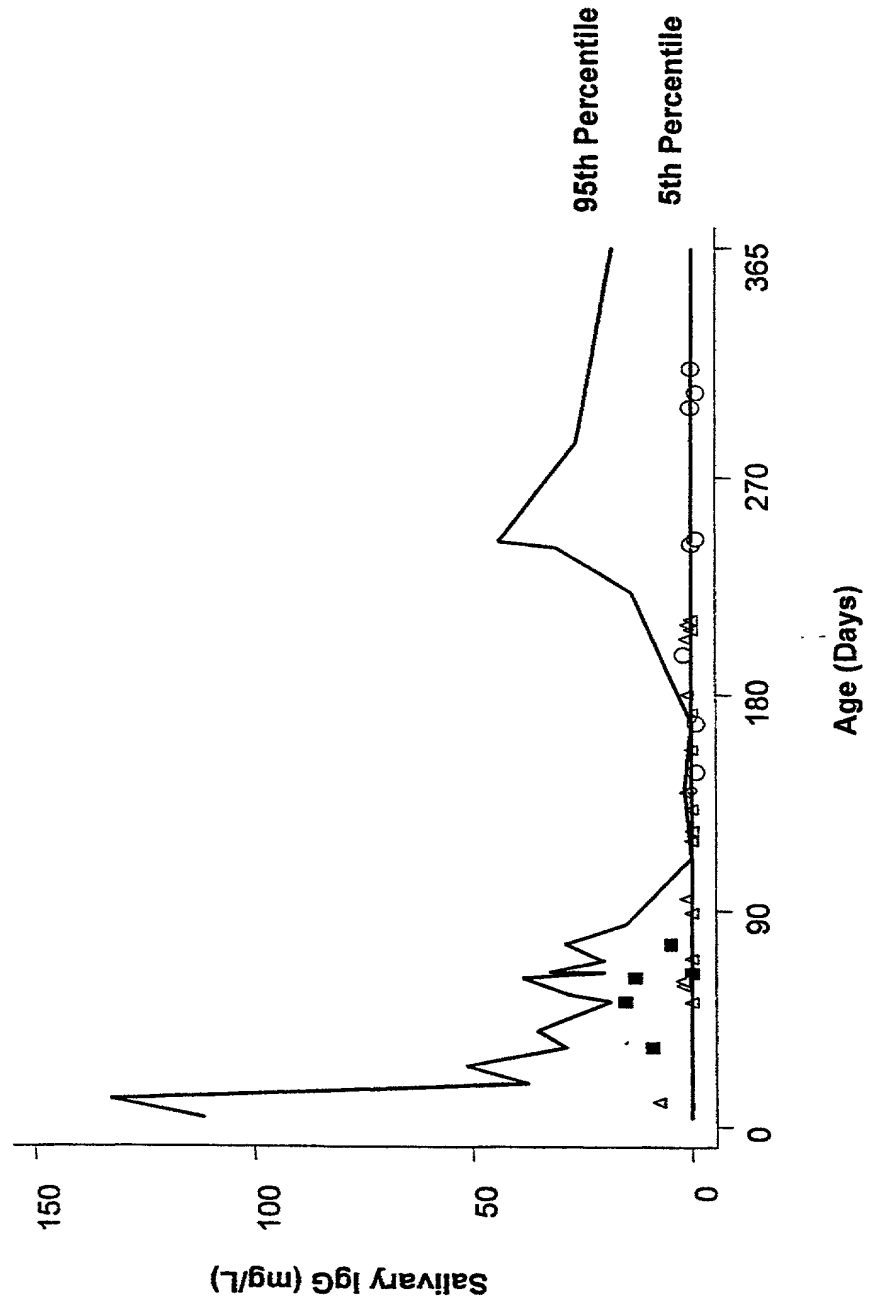


Figure 4

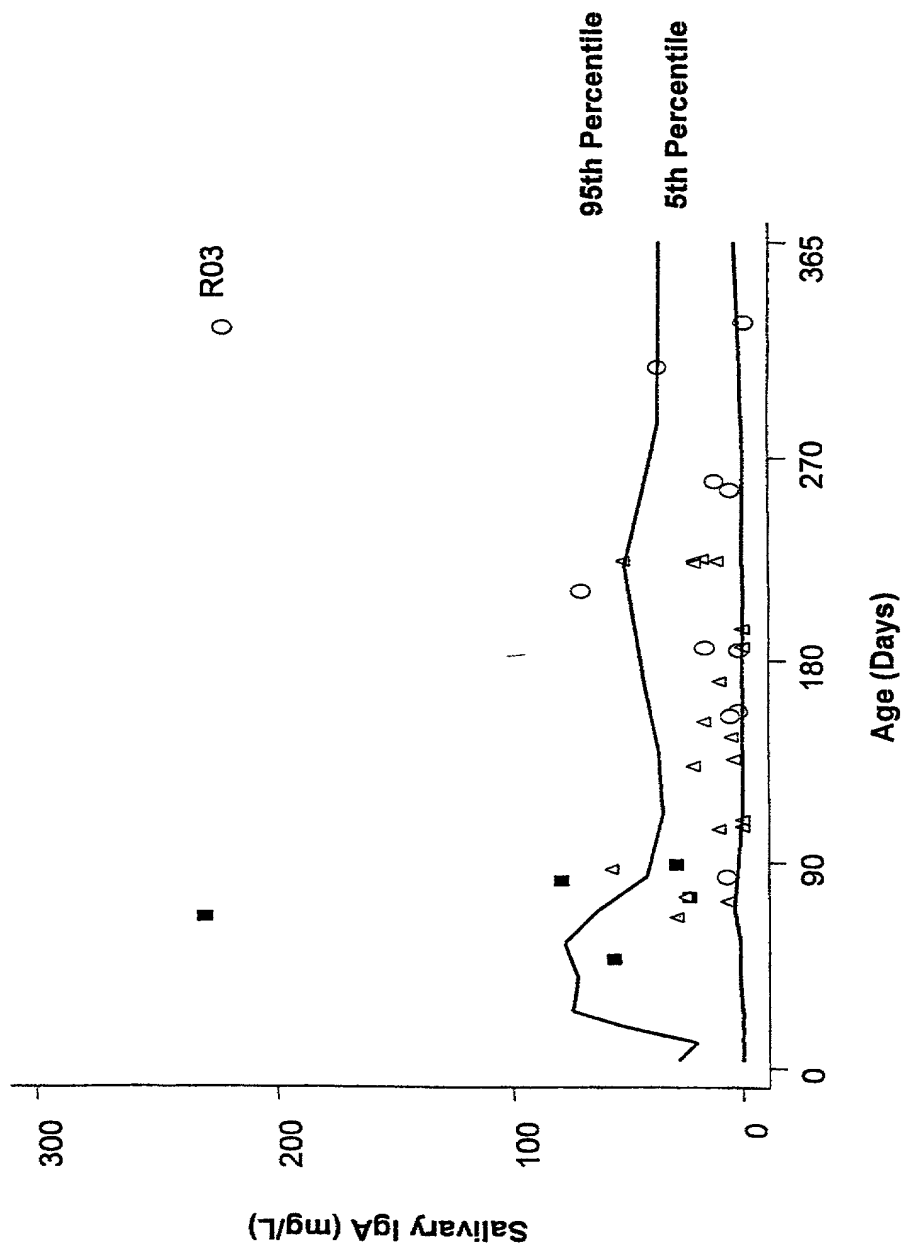


Figure 5

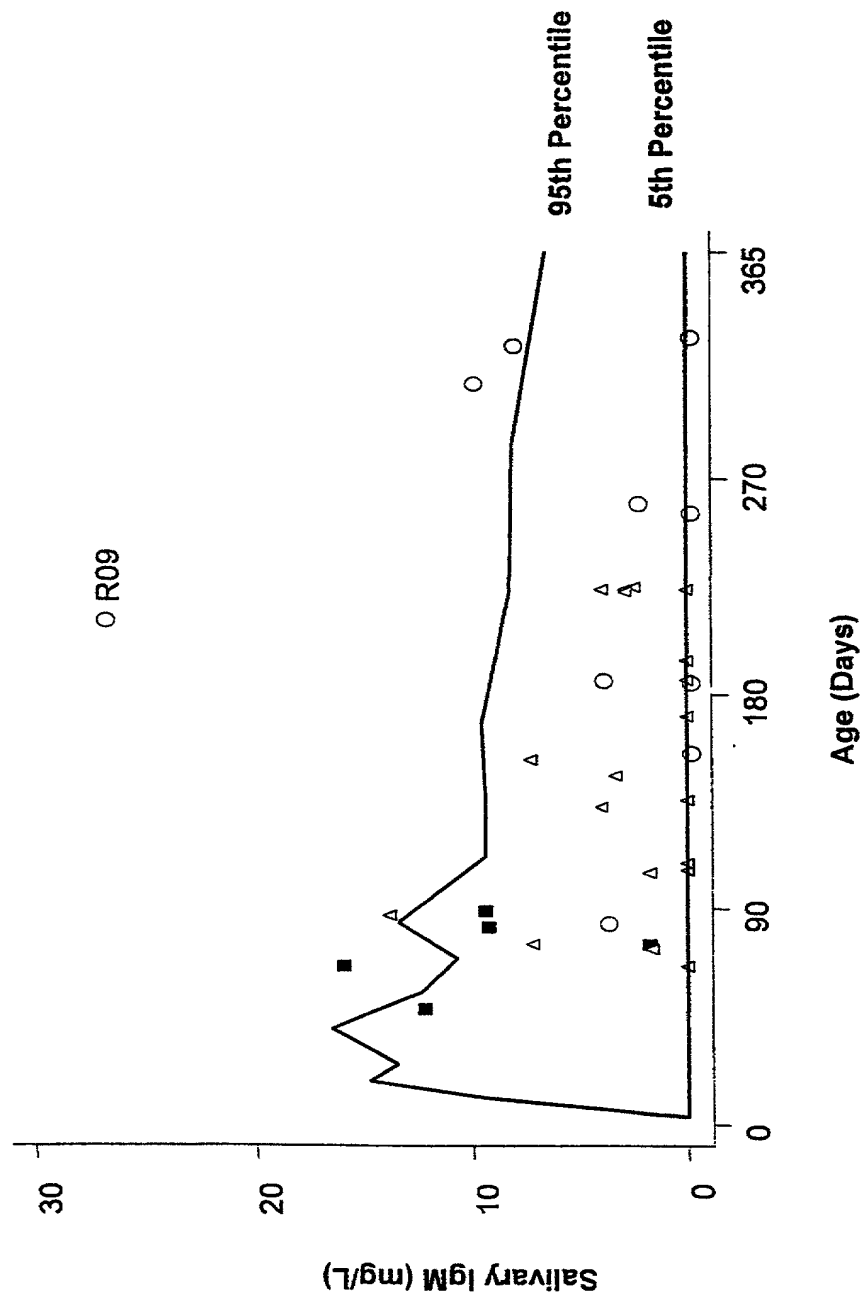
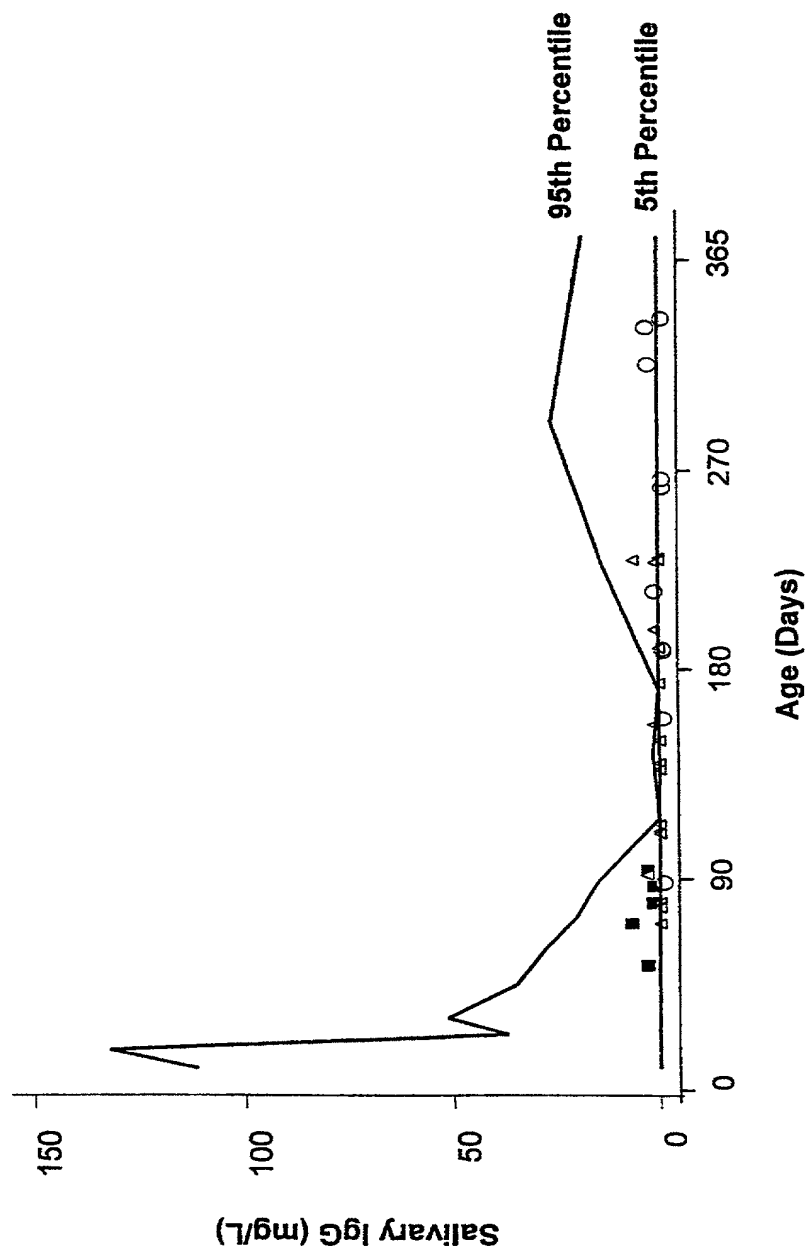


Figure 6



7/9

Figure 7A

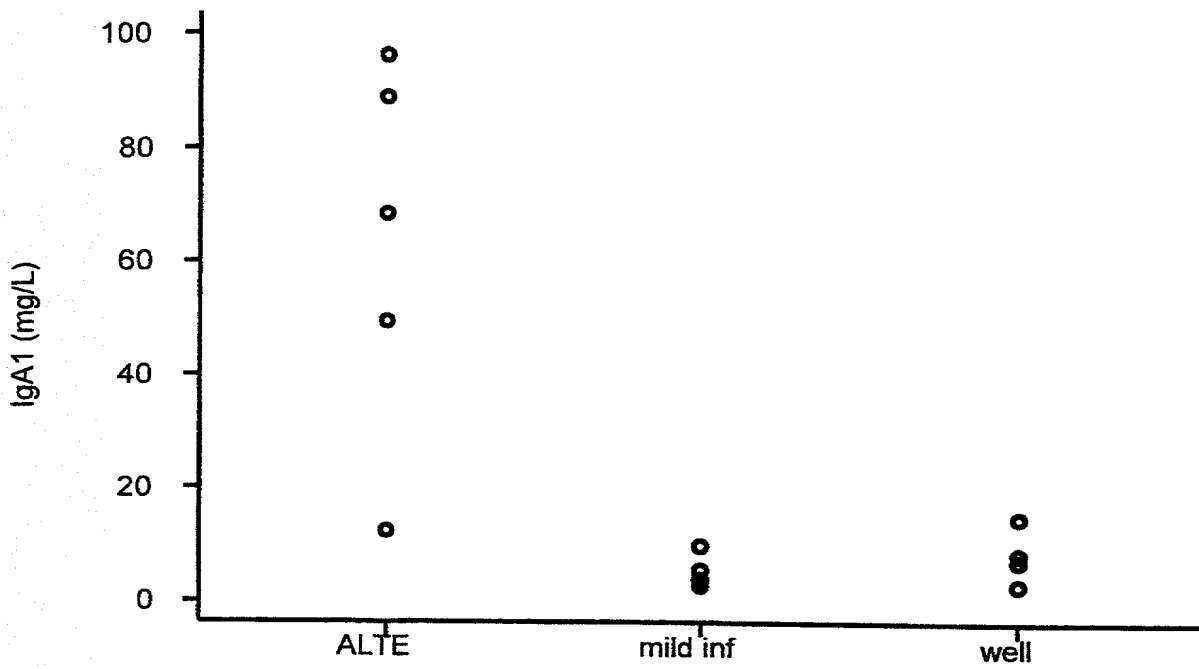
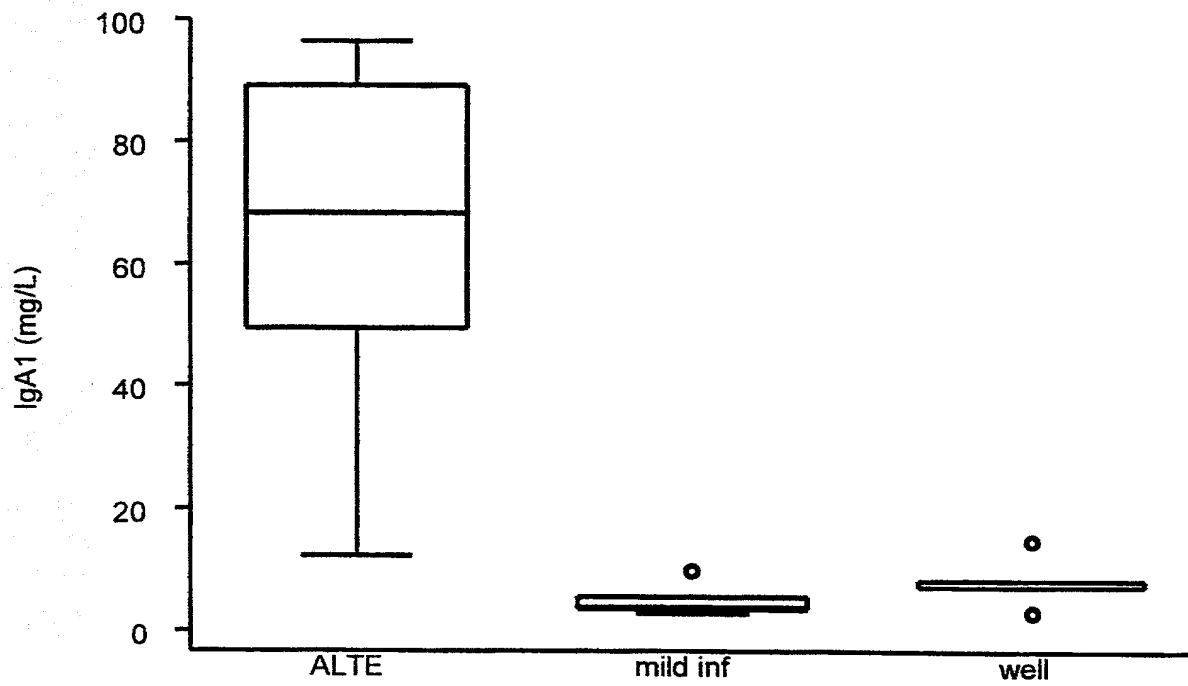
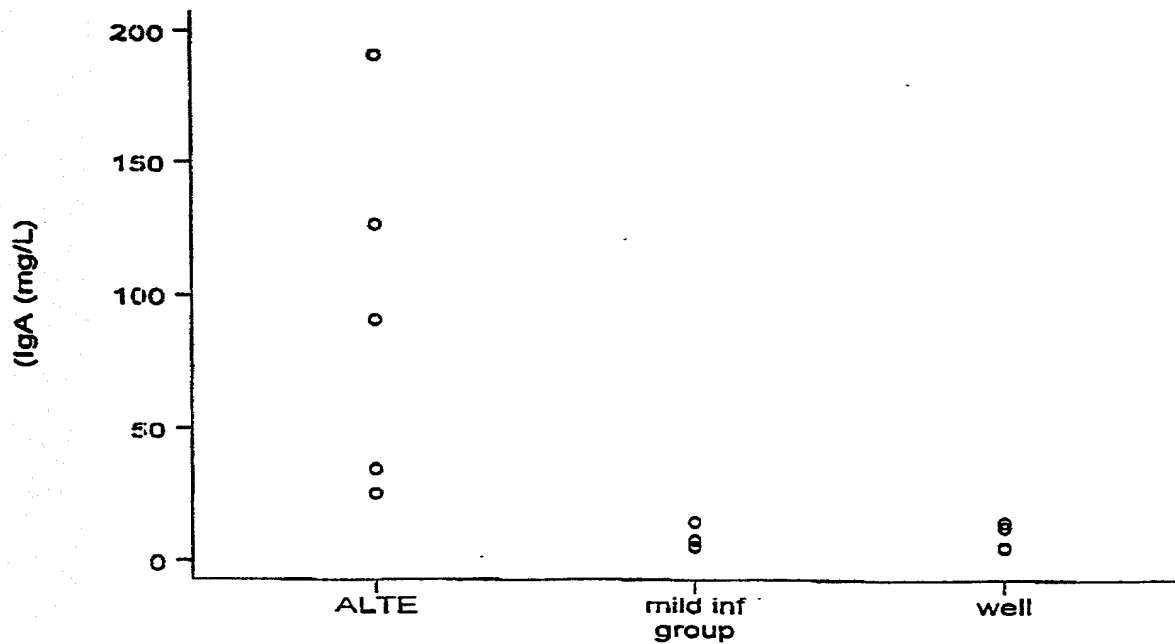


Figure 7B



9/9

Figure 8



As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

the specification of which was filed on December 7, 2001 as 35 USC 371 Serial No. 10/018,127, on June 7, 2000 as International Patent Application No. PCT/AU00/00643; and was filed on June 7, 1999 as Australian application PQ 0810.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in Title 37, Code of Federal Regulation, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Claimed

<u>PCT/AU00/00643</u> (Number)	<u>International</u> (Country)	<u>07 June 2000</u> (Day/Month/Year Filed)
-----------------------------------	-----------------------------------	---

☒ Yes ☐ No

<u>PQ 0810</u> (Number)	<u>Australia</u> (Country)	<u>07 June 1999</u> (Day/Month/Year Filed)
----------------------------	-------------------------------	---

☒ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States Provisional application(s) listed below.

(Application Number)	(Filing Date)
1	1/1/2020
2	1/1/2020
3	1/1/2020
4	1/1/2020
5	1/1/2020
6	1/1/2020
7	1/1/2020
8	1/1/2020
9	1/1/2020
10	1/1/2020
11	1/1/2020
12	1/1/2020
13	1/1/2020
14	1/1/2020
15	1/1/2020
16	1/1/2020
17	1/1/2020
18	1/1/2020
19	1/1/2020
20	1/1/2020
21	1/1/2020
22	1/1/2020
23	1/1/2020
24	1/1/2020
25	1/1/2020
26	1/1/2020
27	1/1/2020
28	1/1/2020
29	1/1/2020
30	1/1/2020
31	1/1/2020
32	1/1/2020
33	1/1/2020
34	1/1/2020
35	1/1/2020
36	1/1/2020
37	1/1/2020
38	1/1/2020
39	1/1/2020
40	1/1/2020
41	1/1/2020
42	1/1/2020
43	1/1/2020
44	1/1/2020
45	1/1/2020
46	1/1/2020
47	1/1/2020
48	1/1/2020
49	1/1/2020
50	1/1/2020
51	1/1/2020
52	1/1/2020
53	1/1/2020
54	1/1/2020
55	1/1/2020
56	1/1/2020
57	1/1/2020
58	1/1/2020
59	1/1/2020
60	1/1/2020
61	1/1/2020
62	1/1/2020
63	1/1/2020
64	1/1/2020
65	1/1/2020
66	1/1/2020
67	1/1/2020
68	1/1/2020
69	1/1/2020
70	1/1/2020
71	1/1/2020
72	1/1/2020
73	1/1/2020
74	1/1/2020
75	1/1/2020
76	1/1/2020
77	1/1/2020
78	1/1/2020
79	1/1/2020
80	1/1/2020
81	1/1/2020
82	1/1/2020
83	1/1/2020
84	1/1/2020
85	1/1/2020
86	1/1/2020
87	1/1/2020
88	1/1/2020
89	1/1/2020
90	1/1/2020
91	1/1/2020
92	1/1/2020
93	1/1/2020
94	1/1/2020
95	1/1/2020
96	1/1/2020
97	1/1/2020
98	1/1/2020
99	1/1/2020
100	1/1/2020

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint Madeline Baer, Reg. No. 36,437; Steven J. Baughman, Reg. No. P-47,414; Johnny Y. Chen, Reg. No. 46,614; Gregory G. Glover, Reg. No. 34,773; Patricia Granahan, Reg. No. 32,227; David P. Halstead, Reg. No. 44,735; Daniel Hansburg, Reg. No. 36,156; Edward J. Kelly, Reg. No. 38,936; Robert A. Mazzarese, Reg. No. 42,852; Colleen H. McDuffie, Reg. No. 43,788; Robert H. Morse, Reg. No. 25,358; James L. Sigel, Reg. No. 39,029; Wolfgang Stutius, Reg. No. 40,256; Matthew P. Vincent, Reg. No. 36,709; as attorneys/agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Address all telephone calls to Matthew P. Vincent at telephone number (617) 951-7000.

Address all correspondence to: **Customer Id No: 28120**

Patent Group
Ropes & Gray LLP
One International Place
Boston, Ma. 02110-2624

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name): **Robert Llewellyn Clancy**

Inventor's signature: Robert Clancy Date: 10.6.02
 Residence: 11 High Street, Newcastle, New South Wales 2300 Citizenship: Australia AUX
 Post Office Address: Same as above PO Box 991 Newcastle 2300 NSW Australia

Full name of second inventor (given name, family name): Maree Gleeson

Inventor's signature: M. Sullivan Date: 10.4.02
Residence: 202 Merewether Street, Merewether, New South Wales 2291, Australia Citizenship: Australia ALX
Post Office Address: Same as above